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Draft Genome Sequences of Six *Bacillus* Strains and One *Brevibacillus* Strain Isolated from the Rhizosphere of Perennial Ryegrass (*Lolium perenne*)

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ABSTRACT Seven strains of endospore-forming bacteria with antagonistic activity against a series of plant pathogens were sequenced in order to investigate their antimicrobial gene clusters and antimicrobial modes of action. The selected strains include six *Bacillus* strains and one *Brevibacillus* strain.

Plant diseases are major yield-reducing and quality-limiting factors in the production of food. More than 50,000 pathogenic species of microorganisms are invasive to crops and cause a yield reduction of about 7.5% (1). To control crop pathogens, many chemical pesticides have been developed and applied, resulting in serious environmental problems. Hence, there is an urgent need to develop sustainable approaches to control crop pathogens. The application of beneficial microbes has been reported to be effective in pathogen control and is environmentally friendly (2, 3). A well-known beneficial genus of bacteria is *Bacillus*, the species of which are Gram positive, rod shaped, spore forming, and widely distributed in the environment. *Bacillus* is able to produce an array of antimicrobial compounds, including surfactins, fengycins, and iturins, which have strong inhibitory activity on different pathogens (4). The ability to form endospores under biotic and abiotic conditions makes various species of *Bacillus* promising commercial biocontrol agents.

Seven *Bacillus*-like biocontrol strains were isolated from the rhizosphere of healthy perennial ryegrass in Groningen, the Netherlands. Briefly, 1 g of rhizosphere soil sample was suspended in 9 ml of 10 mM MgSO₄ solution. After heating at 80°C for 10 min, the soil solution was diluted 1,000 times with 10 mM sterilized MgSO₄ solution and spread onto Lennox broth agar (Formedium) plates. The plates were incubated at 28°C for 24 h to obtain single colonies.

For genome sequencing, a single colony of each strain was grown in 3 ml Lennox broth (Formedium) at 28°C. Overnight cultures were diluted 50-fold in fresh LB broth and grew until the late exponential growth phase. Cells were collected by centrifugation at 10,000 rpm for 2 min, and total DNA was isolated with a GenElute bacterial genomic DNA kit (Sigma-Aldrich) according to the manufacturer's protocol. The draft genomes were determined at GATC Biotech (Germany) with an Illumina HiSeq sequencing system. A total of 5 million paired reads (150 bp) were generated. FastQC version 0.11.5 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to examine the quality of the reads, and low-quality reads were removed with Trimmomatic version 0.38 (5). The reads were assembled *de novo* using SPAdes version 3.11.1 with default parameters (6). All of the genomes sequenced exceeded 150× coverage, and the characteristics of the obtained assemblies and genome features are described in Table 1. The genomes were annotated with the Rapid Annotations using Subsystems Technology (RAST) server (7). Strains were identified to be *Bacillus* and *Brevibacillus* species (Table 1) by phylogenetic analysis with available whole-genome sequences (our unpublished data). Genome mining was conducted with BAGEL4 (8) and antiSMASH (9),

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TABLE 1 Genome features and GenBank accession numbers of the strains

Strain	Genome size (kb)	G+C content (%)	N_{50} (kb)	No. of contigs	GenBank accession no.
<i>Bacillus subtilis</i> MG27	4,188	43.4	1,048	13	QJJA000000000
<i>Bacillus velezensis</i> MG33	4,213	45.7	342	31	QJJB000000000
<i>Bacillus velezensis</i> MG43	3,923	46.5	882	23	QJJC000000000
<i>Bacillus pumilus</i> MG52	3,804	41.6	212	39	QJIZ000000000
<i>Brevibacillus laterosporus</i> MG64	5,153	40.7	130	103	QJJD000000000
<i>Bacillus altitudinis</i> MG75	3,865	41.0	248	42	QIMF000000000
<i>Bacillus pumilus</i> MG84	3,657	41.5	167	33	QJJE000000000

showing various potential novel bacteriocins, nonribosomal peptides (NRPs), and polyketides (PKs) in all seven strains. The exact characteristics and functions of these antimicrobial compounds are under investigation.

Data availability. The genome sequences of the seven potential biocontrol strains have been deposited in DDBJ/EMBL/GenBank under the accession numbers [QJJA000000000](#), [QJJB000000000](#), [QJJC000000000](#), [QJIZ000000000](#), [QJJD000000000](#), [QIMF000000000](#), and [QJJE000000000](#). The versions described in this paper are the first versions, [QJJA010000000](#), [QJJB010000000](#), [QJJC010000000](#), [QJIZ010000000](#), [QJJD010000000](#), [QIMF010000000](#), and [QJJE010000000](#). The raw reads were submitted to the Sequence Read Archive under the accession numbers [SRR8305992](#), [SRR8316559](#), [SRR8316560](#), [SRR8316751](#), [SRR8316749](#), [SRR8316750](#), and [SRR8316752](#).

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REFERENCES

- Nellemann C. 2009. The environmental food crisis: the environment's role in averting future food crises: a UNEP rapid response assessment. UNEP/Earthprint, PBL Netherlands Environmental Assessment Agency, The Hague, the Netherlands.
- Van Loon L. 2007. Plant responses to plant growth-promoting rhizobacteria. *Eur J Plant Pathol* 119:243–254. <https://doi.org/10.1007/s10658-007-9165-1>.
- Kloepper JW, Ryu C-M, Zhang S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94:1259–1266. <https://doi.org/10.1094/PHYTO.2004.94.11.1259>.
- Ongena M, Jacques P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol* 16:115–125. <https://doi.org/10.1016/j.tim.2007.12.009>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP. 2018. BAGEL4: a user-friendly Web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Res* 46:W278–W281. <https://doi.org/10.1093/nar/gky383>.
- Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de Los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0: improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* 45:W36–W41. <https://doi.org/10.1093/nar/gkx319>.